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Life Cycle and Cytogenetic Study of Mosquitoes (Diptera: Culicidae)

Sahar Abd

Abstract

Mosquitoes fall into the Culicidae family of the order Diptera within class Insecta and members of the phylum Arthropod. This family includes two important medical and veterinary *important* disease vectors due to their roles for transmission of various viruses, bacteria, and parasites—Anophelinae and Culicinae. The mosquitoes undergo four stages of transformation during their lifetime: egg, larva, pupa, and adult. These have complete metamorphoses or so called Holometabola. Commonly known as the southern house mosquito, *Culex quinquefasciatus* Say is a medium-sized brown insect that exists throughout the tropics and the lower latitudes of temperate regions, and a vector of many pathogens of humans as well as both domestic and wild animals. Although an intensified interest in mosquito cytogenetics in the past decade has produced a number of contributions to knowledge on this subject, the available information is still superficial and limited to a few mosquito species only. Therefore, the karyotype of the populations of the mosquito *C. quinquefasciatus* has been studied collected from three provinces: Babylon, Baghdad, and Wasit of Iraq. The study showed that the chromosomes karyotyping of this species consisted of three pairs of chromosomes (i.e., $2n = 6$). In conclusion, it is stressed that prospects are especially good for evolutionary and genetic studies involving chromosomal polymorphism.

Keywords: mosquitoes, Diptera, *Culex*, *Aedes* and *Anopheles*

1. Introduction

Female mosquitoes attack humans and animals to obtain the blood necessary to sustain their vital activities and make eggs. They use all warm-blooded animals, causing severe uncomfortable and serious harm due to loss of blood, itching, and allergies as well as transmission of pathogens [1].

Carbon dioxide, which is emitted from respiration as well as sweat, is an attractive substance for mosquitoes [2]. The symptoms or reactions caused by mosquito bites are pink rash around the attacked spot accompanied by itching and pain, and may be accompanied by symptoms of allergies. The saliva of mosquitoes contains chemical components which cause these symptoms on the hosts. *Staphylococcus aureus* found in the salivary glands secretes antigenic substances, agglutinins and anticoagulants, and it has been found that the saliva of mosquitoes prevents the growth of chicken embryos and causes death if infected. Feeding by the mosquito is through the parts of her mouth reaching the capillary blood vessels, which leads to puncture in several areas, causing slow or stop of the blood flow to produce

perfusion and bleeding appears in the form of small bleeding spots, especially in the areas around the eye in children [3].

Mosquitoes attack exposed parts of the nose, ears, and limbs, and there is record of a few hundred *C. quinquefasciatus* mosquitoes per person in 1 h, as *C. quinquefasciatus* prefers blood absorption from the face and trunk [4]. Also found among the areas of the body preferred by mosquitoes for nutrition is the foot more than the leg because the region is characterized by smells resulting from the frequent presence of sweat glands [5].

The nature of mosquito adults in their frequency to a number of families for feeding makes these important to transport pathogens from the infected person or animal to a healthy person or animal [6]. The mosquitoes occupy an important place among the medical insects carrying dangerous pathogens that lead to human death. The most serious of these causes are the cases of malaria, which is due to the species of *Plasmodium* that is transmitted by the female sex species. The *Anopheles* species show a vital transmission of malaria in the world and 380 species of *Anopheles* mosquito have been known as vectors. For malaria, about 60 mosquito species are attracted to humans for nutrition [7].

At least 20 species of malaria pathogens have been registered in South Asia, including India, Pakistan, Bangladesh, and Sri Lanka. The most serious pathogens transmitting species are *Anopheles stephensi* Liston, *Anopheles sacharovi* Favre, and *Anopheles gambiae* Giles [8]. Filariasis lymphatic disease is a serious disease that causes three types of worms: *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* are transmitted by genus *Culex*, *Aedes*, and *Anopheles*. But the most dangerous of these types of worms is *W. bancrofti* [9].

The *Culex* mosquitoes also transmit many pathogens that cause serious diseases to humans, especially those that cause meningitis in humans and animals, and from these initiating West Nile virus and meningitis, *Louis encephalitis virus*, *Japanese encephalitis virus*, and *chicken pox* [10, 11].

Yellow fever, which is a short-term acute illness, often causes death and starts with fever, headaches, and jaundice. The patient's color becomes yellow, internal bleeding and vomiting can occur, and death may take place within 3 days. This type of disease is transmitted by *Culex* and *Aedes* mosquitoes. Other serious diseases whose pathogens are mosquitoes include dengue fever and dengue hemorrhagic fever. The *Aedes aegypti* (Linnaeus), the yellow fever mosquito, and *Aedes albopictus* (Skuse), the Asian tiger mosquito, are the main vectors of these diseases in tropical and subtropical regions. *A. aegypti* is the major carrier of the disease in East Asian and warmer countries [12].

2. Classification of mosquito

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Hexapoda
Class: Insecta
Subclass: Pterygota
Order: Diptera
Suborder: Nematocera
Family: Culicidae
Subfamily: Culicinae [13]

The fragmentary data available on mosquito life cycle and cytogenetics clearly indicate the kind and amount of future work to be done. Out of so many kinds of

mosquitos thus far described, there have been life cycle and cytogenetics information on fewer species and much of this is very superficial. Therefore, the life cycle and the karyotype of the populations of the mosquito *C. quinquefasciatus* have been studied collected from three provinces of Iraq.

3. Life cycle of mosquito

Mosquitoes fall in the Culicidae family of the Diptera order. This family includes two medically important subfamilies: Anophelinae and Culicinae [14]. The Culicidae family has about 3500 species of mosquitoes belonging to 43 genera, including *Culex* [15]. *Culex* is a cosmopolitan genus and one of the largest groups of the family Culicidae with 768 species divided among 26 subgenera [16]. Classification under the *Culex* is based on the diagnostic characteristics of females and males mosquito.

However, most of the available classification keys should be used with caution because the phenotypic traits of females may be either multiform or overlapping. The morphological characteristics of the fourth larval stage are also used to determine species; however, some overlaps between species may also be difficulties for the researchers to obtain an accurate classification of mosquito species. Despite the significant phenotypic similarity between the *northern house mosquito* or *common house mosquito* *Culex pipiens* Linnaeus complex, they differ widely in behavior, physiology, and preference.

Mosquitoes are widespread in all the tropical and subtropical regions of the world, which extend into the Arctic Circle but are absent in Antarctica [17]. The eggs from temperate breeds have more strain than those found in warmer regions [18]. Mosquitoes are found at a height of 550 m and a depth of 1250 m below sea level [19]. The shallow-water marshes containing plants are a preferred environment for the growth and reproduction of mosquitoes. The most important species that prefer these environments are the types of *Culex*, especially *C. pipiens* and *C. salinarius* Coquillett [20].

Most mosquitoes mate shortly after leaving the envelope of the pupa. The sperms are passed by the male and are saved by the female within the spermatheca and all eggs can be fertilized in the female throughout her life. Therefore, one mating is sufficient for each female throughout her lifetime [7].

Female mosquitoes need to obtain the blood meal necessary for egg growth and maturity. This is referred to as the development of anautogenous development, as in the case of type *C. quinquefasciatus*, but in some species where at least the first meal of eggs can develop. Probably, following the payments without a blood meal, the process is called autogenous development as in *Culex molestus* Forskal [15].

Female often need a blood meal either before or after mating before they mature. Many species draw on humans to get their meals from the blood and feed a little on human blood by preference for any other animal so these are called anthropophilic species in their dietary habits, while those that feed primarily on animals (mammals, reptiles, and birds) are animal lover zoophilic, and mosquitoes feeding on birds are called bird lovers ornithophilic [21].

Some mosquitoes prefer feeding at specific times of the day, such as dusk, dawn, or midnight, but daytime feeding can also occur. A few mosquitoes enter homes often for human feeding and are called endophagic feeders in their eating habits, while those that bite outside of their homes are called exophagic [7].

All mosquito species go through four stages during their life cycles (**Figure 1**):

- egg—hatches when exposed to water;

- larva—“wiggler” lives in water and most species come to surface to breathe air;
- pupa—“tumbler” does not feed and it is the stage just before emerging as an adult; and
- adult—flies short time after emerging and after its body parts have hardened.

The mosquitoes undergo four stages of transformation during their lifetime: egg, larva, pupa, and adult. These have complete metamorphoses or are called Holometabola (**Figure 1**). Females usually mate once but put eggs in several batches throughout their lives, the largest of which is the first batch. For a female to do so, she must feed more than once on the blood. The blood meal is digested 2–3 days in the tropics and more so in the temperate regions. Pregnant females look for suitable place to lay eggs. They feed on the blood to put another egg batch. This process is repeated until the mosquito dies, which may last up to 1 month. Eggs are usually brown and long cylindrical, placed vertically on the surface of the water and laid together to form an egg raft, which can contain 30–300 eggs (**Figure 2**). The *Aedes* females lay eggs individually on wet places and eggs hatch usually after 24–30 h. The larva (**Figure 3**) passes through four stages where it is hatched and molts three times, ranging from 1 to 3 days. The larva feeds in minutes amount

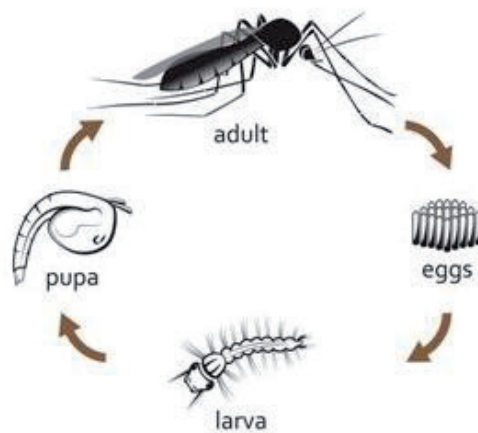


Figure 1.
Life cycle of mosquito.



Figure 2.
Egg raft of Culex quinquefasciatus.

of organic matter as well as on bacteria, primates, lichens, fungi, and spores by means of a permanent circular motion around the mouth through mouth-like mouthwashes.

The larvae are transformed into the pupa and are of the type of active pupa (**Figure 4**), which differ in their appearance from the larvae. The large cephalo-thorax region includes the head and its tail represents the belly of the complete insect. The tail ends with a pair of paddles. Within water, the weight of the pupa is lighter than the weight of water, so they rise again to the surface of the water slowly through the raft or float. The pupa has a pair of trumpets respiratory connected directly to the open air and is used to breathe [11].



Figure 3.
Larva of Culex quinquefasciatus.



Figure 4.
Pupa of Culex quinquefasciatus.

The adult's length is between 3.96 and 4.25 mm (**Figure 5**), and it is made up of a head with two large compound eyes, a thorax, a pair of scaled wings, and six jointed legs. The thorax, legs, and wings are covered with dark brown scales. The abdomen is covered with black scales and some of which are white, and the anal cercus is withdrawn. The adults rest on the surfaces in such a way that the thorax and head are parallel to the surface and the hose may form a small angle with the surface. The antenna of the male is plumose, easily visible near the eye and the antenna of the female is pilose [14].

Male mosquitoes last less than a week and the females may live for a couple of months and that is only with ideal conditions depending on how much warmth and moisture. Sometimes females have to wait for about a day for their reproductive parts to develop completely.

The role of mosquitoes life cycle to manage their populations is vital throughout the world and especially in the tropics because these spread many diseases. Generally, mosquitoes control operations are targeted against three different problems; nuisance mosquitoes bother people around homes, in parks, and recreational areas; economically important mosquitoes reduce real estate values consisting of land and the buildings, adversely affecting tourism and related business interests; and public health is the focus when mosquitoes are vectors or transmitters of infectious diseases [22–27]. Therefore, mosquitoes prevention and control involves a basic understanding of their life cycle, removal of potential egg laying sites, removal of any sources of standing water, keeping of weeds and other vegetation mowed and trimmed to minimize shelter for adult mosquitoes, considering stocking of mosquitoes eating fish for areas with ponds, control of adult mosquitoes, and personal protection [28–38].



Figure 5.
Female adult of Culex quinquefasciatus.

4. Cytogenetic study

During the last so many years, there has been a renewed emphasis upon basic research in mosquito biology. The dramatic development of resistance over the world and the unequivocal demonstration of the genetic basis of this resistance have underlined the need for more research and increasingly intensive research on the genetics and cytogenetics of mosquitoes, and today, relatively little is known in this field.

The study shows that the chromosomes karyotyping of this species consists of three pairs of chromosomes (i.e., $2n = 6$). There are one pair of sexual chromosomes and two pairs of autosomal chromosomes (**Figure 6**).

The average length for autosomal chromosomes collected from Baghdad province is 5.87 and 6.99 μM for chromosome 2 and chromosome 3, respectively, and the length of sexual chromosome is 4.46 μM , the average length for autosomal chromosomes collected from Babylon province is 5.51 and 6.96 μM for chromosome 2 to chromosome 3, respectively, and the length of sexual chromosome is 4.33 μM ; while the average length for autosomal chromosomes collected from Wasit province is 5.5 and 6.97 μM for chromosome 2 and chromosome 3, respectively, and the length of sexual chromosome is 3.89 μM (**Table 1**).

When measuring the arm ratio for the autosomal and sexual chromosomes, it shows that the metacentric chromosomes show the average arm ratio for the chromosomes of the Baghdad province as 1.21, 1.28, and 1.25 μM for chromosomes 1, 2, and 3, respectively; the ratio of arm to autosomal and sexual chromosomes of Babylon as 1.18, 1.15, and 1.20 μM from chromosome 1 to chromosome 3, respectively; and the ratio of arm of the autosomal and sexual chromosomes of the Wasit province as 1.27, 1.20, and 1.30 μM from chromosome 1 to chromosome 3, respectively (**Table 2**).

In regard to the relative length for the autosomal chromosomes, the average length of the relative chromosomes of the Baghdad is 33.98 and 40.18% for chromosome 2 and chromosome 3, respectively, and the relative length of the sexual chromosome is 25.8%, while the relative length of the chromosomes of the Babylon is 32.32 and 41.42% in the case of chromosome 2 and chromosome 3, respectively; the relative length of the sexual chromosome is 25.77%. As for the Wasit province, the relative length of chromosomes is 33.61 and 42.6% in case of chromosome 2 and chromosome 3, respectively, and the rate of relative length of sexual chromosome is the 23.78% (**Table 3**).

When measuring the average ratio of centromere index for this insect of Baghdad province, it is 43.8 and 44.35 μM for chromosome 2 and chromosome 3, respectively, while the average ratio of the centromere index is 45.1 μM for the sexual



Figure 6.
Three pairs of chromosomes of Culex quinquefasciatus.

Region	Ch. No.		
	Ch. I [*] M ± S.D	Ch. II ^{**} M ± S.D	Ch. III ^{**} M ± S.D
Baghdad	4.46 ± 1 a	5.87 ± 1 a	6.99 ± 1 a
Babail	4.33 ± 1 a	5.51 ± 1.52 a	6.96 ± 1 a
Wasit	3.89 ± 1 a	5.5 ± 1.52 a	6.97 ± 1 a
	P = 0.7721	P = 0.7793	P = 1.0
[*] <i>Sexual chromosomes.</i> ^{**} <i>Mean autosomal chromosomes.</i>			

Table 1.
The average total length (μM) of chromosomes collected from different province in Iraq.

Region	Ch. No.		
	Ch. I [*] M ± S.D	Ch. II ^{**} M ± S.D	Ch. III ^{**} M ± S.D
Baghdad	1.21 ± 1 a	1.28 ± 1 a	1.25 ± 1 a
Babail	1.18 ± 1 a	1.1 a	1.20 ± 1 a
Wasit	1.27 ± 1 a	1.20 ± 1 a	1.30 ± 1 a
	p = 0.99	p = 0.99	p = 0.99
[*] <i>Sexual chromosomes.</i> ^{**} <i>Mean autosomal chromosomes.</i>			

Table 2.
The average arm ratio for the chromosomes of the different provinces in Iraq.

Region	Ch. No.		
	Ch. I [*] M ± S.D	Ch. II ^{**} M ± S.D	Ch. III ^{**} M ± S.D
Baghdad	25.8% ±10 a	33.98% ±6.08 a	40.18% ±8.54 a
Babail	25.77% ± 10 a	32.79% ±10 a	41.42% ±8.54 a
Wasit	23.78% ± 10 a	33.61% ±10 a	42.6% ±10 a
	p = 0.96	p = 0.83	p = 0.99
[*] <i>Sexual chromosomes.</i> ^{**} <i>Mean autosomal chromosomes.</i>			

Table 3.
The average length of the relative chromosomes of the Culex quinquefasciatus.

chromosome. The average ratio of the centromere index of the Babylon insect is 46.5 and 46.6 μM for chromosome 2 and chromosome 3, respectively, while the chromosome reached 45.72 μM for the sexual chromosome, and the average centromere index of the Wasit province is 45.1 and 43.32 μM for chromosome 2 and chromosome 3, respectively, and for sexual chromosome, it is 44 μM (Tables 4 and 5).

Region	Ch. No.		
	Ch. I [*] M ± S.D	Ch. II ^{**} M ± S.D	Ch. III ^{**} M ± S.D
Baghdad	45.1% ± 10 a	43.8% ± 10 a	44.35% ± 10 a
Babail	45.72% ± 10 a	46.5% ± 10 a	46.6% ± 10 a
Wasit	44% ± 10 a	45.1% ± 10 a	43.32% ± 10 a
	p = 0.924	p = 0.980	p = 0.951
[*] Sexual chromosomes.			
^{**} Mean autosomal chromosomes.			

Table 4.
The average ratio of centromere index chromosomes of the *Culex quinquefasciatus*.

Region	Ch. No.		
	Ch. I [*]	Ch. II ^{**}	Ch. III ^{**}
Baghdad	M Metacentric	M Metacentric	M Metacentric
Babail	M Metacentric	M Metacentric	M Metacentric
Wasit	M Metacentric	M Metacentric	M Metacentric

SM: arm ratio = 1.50–2.99. Centromere index = 40.0–25.1.
M: arm ratio = 1.00–1.49. Centromere index = 50.0–40.1.
^{*}Sexual chromosomes.
^{**}Autosomal chromosomes.

Table 5.
Classification of chromosomes of the *Culex quinquefasciatus* from some regions of Iraq by arm ratio and centromere index by Levan et al. [38].

These results provide important genetic information for understanding the chromosomal structure of *C. quinquefasciatus* mosquito from some regions of Iraq. This work opens the ways toward the creation of useful in detail studies of the chromosomes in additional mosquito species.

5. Conclusion

While all mosquitoes need standing water to reproduce, some of the other species have evolved so specifically that they will only lay their eggs in natural or artificial containers. The larvae of most mosquito species hang suspended from the water surface because they need air to breath. An air tube, called a siphon, extends from the larva’s posterior to the water surface. Pupae are also physically active and employ a rolling or tumbling action to escape to deeper water, which is why they are commonly referred to as “tumblers.” The pupal stage lasts from 1.5 to 4 days, after which the pupa’s skin splits along the back allowing the newly formed adult to slowly emerge and rest on the surface of the water. The present study shows that the chromosomes karyotyping of *C. quinquefasciatus* species consists of three pairs of chromosomes (i.e., 2n = 6). Certainly much of this information is of a descriptive sort and easy to obtain by present techniques. Life cycle and karyotype studies in additional species can be simply done and should be carried out in a standardized way.

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Author details

Sahar Abd
Department of Biology, College of Science for Women, Baghdad University,
Baghdad, Iraq

*Address all correspondence to: sahar_abd2008@yahoo.co.uk

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